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# *Detection of Chlamydophila psittaci in chickens by complement fixation test and polymerase chain reaction*

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**This study was carried out on 68 randomly collected chickens located at Ras Sedr Research Station, Desert Research Center, 68 serum samples were examined serologically by complement fixation test (CFT). Twenty out of 68 (29.91%) had antibodies against *Chlamydophila psittaci*. Ten blood samples of the serologically positive cases were subjected to polymerase chain reaction (PCR) and showed positive results for *Chlamydophila psittaci* at 119 bp. Therefore PCR was found to be reliable, rapid, sensitive and specific technique for the detection *Chlamydophila psittaci* in birds. Serologically positive birds did not show any clinical symptoms of disease, but they were in contact with sheep and goat that showed previous abortion and were positive for *C. abortus.* It is recommended to avoid breeding of chickens with other animal species in the same yard because chickens become asymptomatic carrier with shedding of *Chlamydophila psittaci* in their feaces and respiratory discharges.**

Chlamydia, are obligate intracellular parasites causing a variety of infections in animals and birds as well as respiratory, genital, digestive and ocular infections in human (Duan *et al*., 1999; Ketz and Carpenter, 1999). Chlamydiosis is a very important infectious disease in more than 469 different bird species (Kaleta and Taday, 2003). In addition chlamydial infections have also been reported in mammals, reptiles and amphibians (Eugster, 1980; Wilcke *et al*., 1983; Vanrompay *et al*., 1994).

Everett *et al*. (1999) proposed a new classification of chlamydia into two genera and nine species, based primarily on ribosomal RNA sequence, also chlamydiosis in birds, has been recently renamed chlamydophilosis.

The organism is shed in the feces and respiratory secretions of infected birds which play an important role in the transmission of infection. Other birds pick up the organism by inhaling contaminated aerosol. Although infected birds may become extremely ill and die, most birds usually become asymptomatic carriers and they act as source of infection. Without specific test, it is difficult to differentiate negative from positive psittacosis carrier (Eugster, 1980).

Serological diagnosis of chlamydial infection in birds can be based on serological evidence coupled with isolation of the etiological agent (Chahota *et al*., 1997).

The diagnosis of *Chlamydia psittaci* infection in birds often requires a multiple test approach in order to assure the most accurate results (Grimes, 1984, 1985; Grimes and Arizmendi, 1990 and Tully, 1991).

In previous studies, using complement fixation test, the presence of *Chlamydophila* spp. in pigeons was confirmed by (Greguric *et al*., 1989; Vlahovic, *et al*., 1998; Pavak *et al*., 2000) as they reported that 43.88%, 40.9% and 47.70% of pigeons were positive for *C. psittaci* antibodies respectively.

Vlahovic *et al*. (1998), Dottori *et al*. (2000), Guscetti *et al*. (2000), Kemf *et al*. (2000) and Travnicek *et al*. (2000) used complement fixation test to detect antibodies against chlamydia in avian sera.

Due to cross reactivity between *Chlamydophila* species the CFT is not specific and polymerase chain reaction (PCR) assays can be used to distinguish *C. psittaci* infection from infection with other *Chlamydophila* species (Messmer *et al*., 1997).

The aim of this study was to conduct serodiagnostic studies on sera of chickens by complement fixation test and polymerase chain reaction (PCR).

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**Table (1): The master mix ingredients and primers concentration used in PCR.**

| Constituent | Initial concentration | Amount (µl) | Final concentration | $x^8$ |
|---|---|---|---|---|
| **Distilled water** | | 13.2 | | 105.6 µl |
| **Buffer** | 10 x | 2.0 | | 16.0 µl |
| **dNTP** | 10mM | 0.4 | 0.2mM | 3.2 µl |
| **Taq polymerase** | 5µ/Ml | 0.4 | 2µ/Ml | 3.2 µl |
| **Primer 2AF** | 20 mM | 1.0 | 1 mM | 8.0 µl |
| **Primer 2Br** | 20 mM | 1.0 | 1mM | 8.0 µl |
| **Total volume** | | 18.0 µl | | 144.0 µl |

**Table (2): results of CFT in sera of chickens.**

| Total No. of chicken's sera | 68 randomly serum samples | | | |
|---|---|---|---|---|
| | Positive | | Negative | |
| Results of CFT | No. | % | No. | % |
| | 20 | 29.91 | 48 | 70.09 |

CFT titer ranged from 1/8 – 1/128

## Materials and Methods

**Chickens.** This study was preformed on randomly collected total number of 68 chickens located in Ras Sedr station (Desert Research Center, Egypt). The history and clinical examination of poultry in farm were recorded.

**Samples.** Blood samples were collected from chickens and sera were used in PCR and serological tests respectively.

**Antisera**. Reference antisera for Chlamydia; *Chlamydia psittaci*, ( Seiken, Denka Seiken Co., LTd, Tokyo, Japan) was used in CFT for detection of chlamydia bodies in the suspected materials.

**Reference Chlamydia antigen.** It was obtained from Denka Seiken Co., LTd, Tokyo, Japan. It was used for serological detection of antibodies.

**Complement.** Freeze dried preparation of preserved guinea pig serum (Welcome) was used in complement fixation technique.

**Polymerase Chain Reaction (PCR).** Ten randomly collected blood samples from serologically positive cases were subjected to PCR.

**DNA extraction.** The genomic DNA was extracted from samples using Invisorb Spin Blood Mini Kit (Invitek GmbH Gesellschaft Biotechnik-Robert-Rossle-Berlin).

PCR amplification of chlamydial DNA. PCR amplification of chlamydial DNA was performed on DNA extracted from serum samples using oligonucleotide primers Chla.2 AF:5-GCTTTTCTAATTTACACC-3 and Chla. 2 Br: 5- ATAGGGTTGAGACTATCCACT - 3 according to (Sykes *et al.,* 1997). 2 µl of template added to each tube containing master mix (Table.1). Distilled water was used as negative control while pure DNA of Chlamydia psittaci was used as positive control. The reaction was subsequently at 95°C for 10 min. then for 40 cycles at 95°C for 30 seconds, 50°C for 30 seconds, and 72°C for 45 seconds, followed by an additional elongation at 72°C for 10 minutes. Reaction product was visualized by ethidium bromide staining under UV transillumination after electrophoresis on 1.5% agarose gel.

## Results

**History of farms and clinical manifestation.** Ras Sedr Research Station contains poultry as well as small ruminant. Some pregnant ewes and pregnant goats aborted at late stage of pregnancy and the chlamydial infection was proven by serological test and PCR. Detection of antibodies against chlamydia and positive PCR results for *Chlamydophila abortus* (*Chlamydia psittaci*) on aborted foeti were also achieved during the present investigation. To investigate the source of infection, seroprevalence and PCR on chicken sera revealed positive results for *Chlamydophila psittaci* (*Chlamydia psittaci*) although these chickens did not show any symptoms of chlamydial infections.

**Serological results.** Results of serological studies were demonstrated in Table 2.

**Results of Polymerase Chain Reaction (PCR).** Ten randomly collected samples of blood of chickens from serologically positive cases for *Chlamydophila psittaci* revealed positive results by using PCR at 119 bp. The positive control showed the excepted amplification product

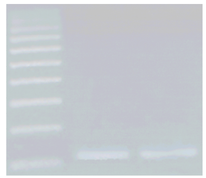



**Fig. (1): PCR of *Chlamydophila psittaci* (*Chlamydia psittaci*) DNA was extracted from blood of chicken. PCR products were separated on 1.5% agarose gel and stained with ethidium bromide lane1, 100 bp ladder marker ; lane 2, positive control (*C. psittaci*); lane 3, specific *C. psittaci* PCR product (119 bp detected).**

(119bp) as shown (Fig.1).

## Discussion

Infections with *Chlamydophila psittaci* are quite often found in commercial poultry (chicken, turkeys, ducks, geese and doves). The spread of the causative organisms from poultry to animal and human beings may happen (Heike, 2004).

Serological study by using complement fixation test is the most commonly used for detection of antibodies against *Chlamydophila psittaci*. Positive results were recorded in 29.91% of chickens' sera. The detection of *Chlamydophila psittaci* antibodies in the investigated samples by CFT is similar to that obtained by (Vlahovic *et al*., 1998; Pavlak *et al*., 2000).

CFT was found to be more sensitive than agar gel precipitation test and elementary body agglutination with 21, 16, 17.5 and 12.5%, respectively in seroprevalence of chlamydiosis (Paul *et al*., 2002).

Although, CFT is commonly used, but is of quite low sensitivity (Schmeer 1983; Gerbermann and JanecZek, 1991; Salinas *et al*., 1993; Bendheim *et al*.,1998; Prunkner-Radovic *et al*., 2005). On the other hand, ELISA in comparison with CFT was found to be more accurate and highly sensitive (Salinas *et al*., 1993).

In this work because CFT is complicated by false positive due to cross reaction between *Chlamydophila* species (Longbotton *et al*., 2001) so using molecular biology for diagnosis of *Chlamydophila psittaci* is very recommended.

Regarding the advanced techniques for the detection of *Chlamydophila psittaci*, ten randomly collected blood samples of chickens serologically positive by CFT were subjected to PCR using both "2A" and "2B" primers which are specific for identification of *Chlamydophila psittaci* DNA. All the examined blood samples showed the expected amplification product specific for *Chlamydophila psittaci* (119 bp). This finding was in parallel line with Heike, (2004) and Dovc *et al*. (2005) who reported that PCR is a specific, sensitive and rapid test for the detection of *Chlamydophila psittaci* in birds.

In this study the chickens were asymptomatically carriers for *Chlamydophila psittaci* in their body excretions as faeces or respiratory discharges. It is concluded that avoidance of breeding of chickens with other animal species in the same yard.

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## استخدام اختبارى التثبيت للمتمم والبلمرة المتسلسل فى تحديد ميكروب الكلاميدوفيلا سيتاسى فى الدواجن

أجريت هذه الدراسة على عدد ٦٨ طائر (من الدجاج) باختيار عشوائى فى محطة رأس سدر التابعة لمركز بحوث الصحراء والتي سبق أن ظهر بها مشكلة إجهاضات متكررة فى المجترات الصغيرة وتم تجميع عينات الدم من الطيور وفصل السيرم وخضعت هذه العينات لاختبار تثبيت المتمم وظهرت الأجسام المضادة فى عدد ٢٠ عينة بنسبة (٢٩,٩١%) و٤٨ عينة سالبة بنسبة (٧٠,٠٩%) ونتيجة الخطأ الوارد فى اختبار التثبيت التكميلى نتيجة التداخل بين عائلة الكلاميديا فقد تم عمل اختبار تفاعل البلمرة المتسلسل لعدد عشرة عينات دم ايجابية سيرولوجيا وكانت النتيجة ايجابية لميكروب الكلاميدوفيلا سيتاسى عند وزن جزيئي bp ١١٩ . ولهذا يعتبر اختبار تفاعل البلمرة المتسلسل دقيق وسريع وأكثر حساسية فى تشخيص عدوى الكلاميديا . وقد ثبت من نتائج هذا البحث أن الدواجن تعتبر من حوامل ميكروب الكلاميدوفيلا سيتاسى دون ظهور آى أعراض فى بعض الأحيان وينتشر الميكروب فى الإفرازات البرازية والتنفسية فينصح بعدم تربيتها مع آى حيوانات أخرى حتى لا تتسبب فى نقل العدوى .